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Characterization of the Pheromone Binding Protein PBPLma

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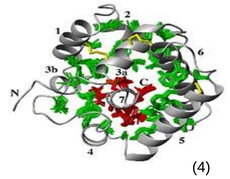
(4)

Introduction

- Pheromone binding proteins (PBPs) play an important role in the sexual behavior of many insects
  - PBPs bind to structurally similar families of pheromones
  - Transport pheromone across aqueous lymph to pheromone receptors (1,2) (Figure 1)

- PBPs may work through a different mechanism at high concentrations of pheromone
  - At high concentrations of pheromone, if all pheromone molecules induced a signal, the pheromone receptor complexes would rapidly become saturated
  - PBPs may form aggregates so that only a small fraction of the molecules are sensed by the receptor
  - Recent research suggests aggregate formation occurs but the exact mechanism remains unknown (3)

- One model is that once a PBP binds a pheromone it is conformationally altered so that it is more likely to bind to other odorant bound PBPs (figure 2)
  - Formation of aggregate of PBPs bound to pheromone
  - Only a small fraction of pheromone would be able to reach the receptor complex

- The PBP PBP*Lma* found in the cockroach *Leucophaca maderae* provides an ideal model to study this system
  - This protein has already been sequenced and purified
  - Small (15 kDa), stable structure
  - Binds the flourophore 8-anilino-1-naphtalenesulphonic acid (ANS)

Goals

- Transform expression strain of *E. coli* with vector containig PBP*Lma*
- Purify protein from *E. coli*
- Characterize monomeric binding using ANS fluorescence assay
- Characterize aggregate formation using native-PAGE and fluorescence anisotropy

Methods

Total RNA extraction
Specific PCR amplification of PBP*Lma*
Restriction and ligation of target and vector
Transformation of *E. coli* with target containing vector
Purify vector from *E. coli* and restrict

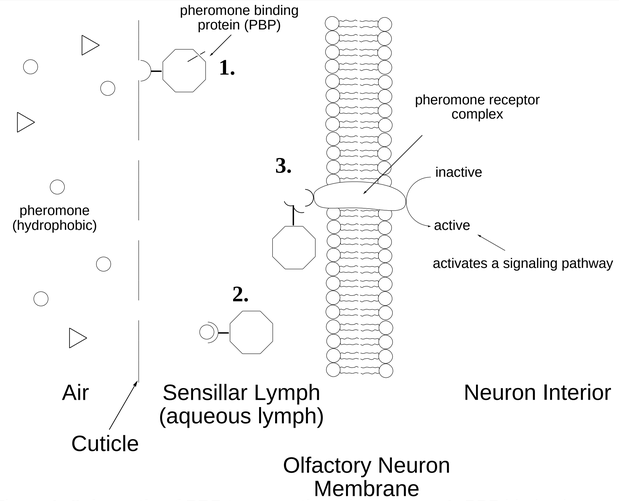

Figure 1. **Schematic of PBP transporting pheromone.** 1. PBP binds pheromone at interface of air and cuticle. 2. PBP transports pheromone through the lymph. 3. PBP presents pheromone to receptor which initiates a signaling pathway.

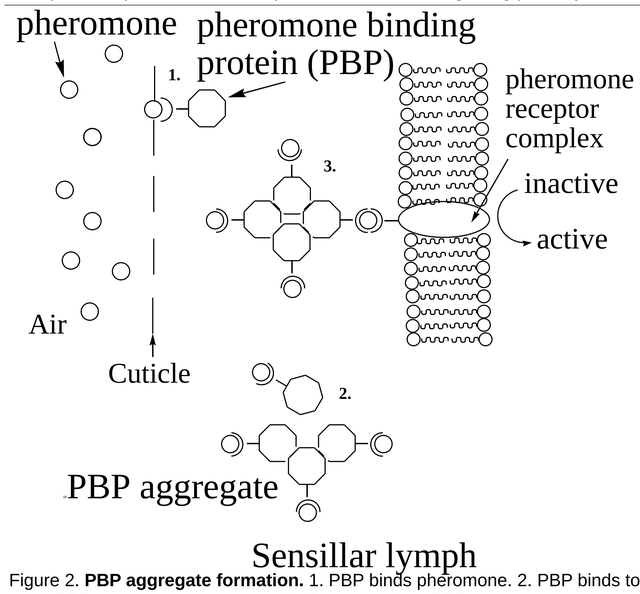

Figure 2. **PBP aggregate formation.** 1. PBP binds pheromone. 2. PBP binds to other PBPs forming an aggregate. 3. Only one of the PBPs in the aggregate is able to present the pheromone to the receptor.

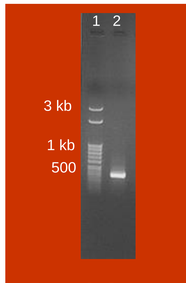

Figure 3. **Gel electrophoresis of RT-PCR product.** (1) DNA ladder (Promega). (2) Product from PCR amplification of RT-PCR product was about 360 bps.

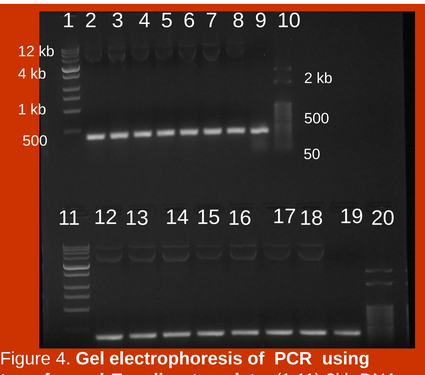

Figure 4. **Gel electrophoresis of PCR using transformed *E. coli* as template**. (1,11) 3kb DNA ladder (10,20) 12 kb DNA ladder (Promega). (2-8,12-18) PCR using purified plasmid from 16 different transformed colonies as the template. The insert is about 360 bp, roughly three times larger than PBP*Lma*.

Results

- Extracted RNA from adult *L. maderae*
- Used RT-PCR with specific primers to amplify cDNA (Figure 3)
- Cloned into p-Gem-T vector (Novagen) and transformed into *E. coli* JM109
- Restricted insert out of p-Gem-T vector using EcoRI and HindIII
- Ligated insert into pET22b+ expression vector (Novagen) and cloned into *E. coli* JM109 (Figure 4)
- Purified the plasmid from transformed colonies and sent to The Ohio State University for sequence analysis
- 5 differences between published sequence and insert (Table 1) -1 deletion and 4 mutations; 2 cause amino acid changes

Future plans: Will repeat cloning process with new primer to eliminate the deletion and will then continue with expression and purification.

Table 1. Sequencing results.

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CGGACTCTACCCAGAGCTACAAGGACGCTATGGGGCCGCTGGTAAGAGAGTGCATGGGCA  121
||||||||||| |||||||||| |||||||||||||||||||||||||||||||||||||
CGGACTCTACC-AGAGCTACAAGGGCGCTATGGGGCCGCTGGTAAGAGAGTGCATGGGCA  212
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deletion — aspartic acid to glycine
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GTGTCTCTGCCACTGAAGACGACTTCAAAACGGTTTTGAACAGGAACCCTCTGGAATCAA  181
||||||| |||||||||||||||||||||||||||||||||||||||||||||||||||
GTGTCTCCGCCACTGAAGACGACTTCAAAACGGTTTTGAACAGGAACCCTCTGGAATCAA  272
```
no change
```
GGACAGCTCAGTGTTTGCTAGCCTGCGCCCTGGACAAGGTGGGCCTTATCTCACCAGAAG  241
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GGACAGCTCAGTGTTTGCTAGCCTGCGCCCTGGACAAGGTGGGCCTTATCTCACCAGAAG  332

GCGCCATCTATACAGGAGATGACCTGATGCCTGTCATGAACCGACTGTACGGTTTCAACG  301
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GCGCCATCTATACAGGAGATGACCTGATGCCTGTCATGAACCGACTGTACGGTTTCAACG  392

ACTTCAAGACAGTCATGAAGGCCAAGGCCGTGAACGACTGCGCCAATCAAGTGAACGGTG  361
|||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||
ACTTCAAGACAGTCATGAAGGCCAAGGCCGTGAACGACTGCGCCAATCAAGTCAACGGTG  452
```
no change
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CGTATCCGGACAGGTGTGATCTTATCAAGAACTTCACCGATTGCGTCCGCAACTCCTACT  421
|||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
CGTATCAGGACAGGTGTGATCTTATCAAGAACTTCACCGATTGCGTCCGCAACTCCTACT  512
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proline to glutamine
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GA  423
||
GA  514
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References

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